
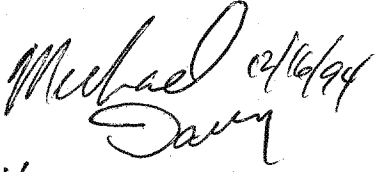




MRID No. 427741-11

## DATA EVALUATION RECORD

1. **CHEMICAL:** Dicamba.  
Shaughnessey No. 029801.
2. **TEST MATERIAL:** Dicamba technical; CAS No. 1918-00-9; Batch No. 52204112; 89.5% active ingredient; a white solid.
3. **STUDY TYPE:** 123-2. Growth and Reproduction of Aquatic Plants - Tier 2. Species Tested: Duckweed (*Lemna gibba*).
4. **CITATION:** Hoberg, J.R. 1993. Dicamba Technical - Toxicity to the Duckweed *Lemna gibba*. SLI Report No. 93-3-4665. Conducted by Springborn Laboratories, Inc., Wareham, MA. Submitted by Sandoz Agro, Inc., Des Plaines, IL. EPA MRID No. 427741-11.
5. **REVIEWED BY:**  
  
Mark A. Mossler, M.S.  
Associate Scientist  
KBN Engineering and  
Applied Sciences, Inc.  
  
Signature:   
Date: 8/13/93  
  
6. **APPROVED BY:**  
  
Pim Kosalwat, Ph.D.  
Senior Scientist  
KBN Engineering and  
Applied Sciences, Inc.  
  
Signature: P. Kosalwat  
Date: 8/13/93  
  
Henry T. Craven, M.S.  
Supervisor, EEB/EFED  
USEPA  
  
Signature:   
Date: 2-22-95  
  
7. **CONCLUSIONS:** This study is scientifically sound and meets the requirements for a Tier 2 aquatic plant growth and reproduction study. Based on mean measured concentrations of dicamba, the 14-day NOEC and LOEC for *L. gibba* were 0.20 and 0.39 mg ai/l, respectively. The 14-day EC<sub>50</sub> was >3.25 mg ai/l, which was greater than the equivalent of the maximum application rate.
8. **RECOMMENDATIONS:** N/A.
9. **BACKGROUND:**



10. DISCUSSION OF INDIVIDUAL TESTS: N/A.11. MATERIALS AND METHODS:

A. Test Species: *Lemna gibba* G3 used in the test came from laboratory stock cultures originally obtained from the University of California, Los Angeles. Stock cultures were maintained in Hoagland's medium (with pH adjusted to 5.0) under continuous 3.8-5.4 klux illumination and a temperature of  $25 \pm 1^\circ\text{C}$ . Lighting was provided by Duro-Test Vita-Lite® fluorescent tubes. Transfers were made into fresh medium once weekly. The plants used in the test were taken from a six-day old stock culture.

B. Test System: Sterile, covered 270-ml crystallizing dishes were conditioned by rinsing with the appropriate solution. One-hundred ml of the appropriate test solution were placed into each dish.

The test was performed in a growth chamber with conditions similar to those used in culturing. Light was provided continuously at an intensity of 3.2-4.8 klux.

C. Dosage: Fourteen-day growth and reproduction test. Based on the results of a range-finding test, five nominal concentrations of 0.25, 0.50, 1.0, 2.0, and 4.0 mg active ingredient (ai)/l were selected for the definitive test. The maximum application rate for dicamba was reported to be 4 lb ai/acre, which is equivalent to 2.9 mg ai/l if applied to a 15-cm water column.

A 400 mg ai/l primary stock solution was prepared by dissolving 0.20 g (as ai) of test material in Hoagland's medium to the final volume of 500 ml. Appropriate volumes of the primary stock solution were diluted to the final volume of 500 ml in Hoagland's medium to prepare the treatment solutions. A medium control was also prepared.

D. Test Design: The test consisted of 3 replicate dishes per treatment level and control. *Lemna gibba* (5 plants with 3 fronds each) was aseptically introduced into each dish within 45 minutes of solution addition. On test days 3, 6, 9, 12, and 14, fronds were counted and observations were made. At initiation and after each counting, the dishes were positioned in the assigned random location in the growth chamber. After terminal

counting, the fronds were dried at 70°C for three days to determine dry weight per replicate.

The pH was measured at test initiation and termination. Temperature was recorded continuously with a minimum/maximum thermometer in a flask of water in the environmental chamber. The light intensity was recorded daily.

At test initiation and termination, samples were removed from each treatment and control solution for analysis by high performance liquid chromatography. A set of three quality control solutions were prepared at test initiation and termination to monitor the precision and quality control during analysis.

- E. **Statistics:** Since no concentration tested inhibited either frond number or dry weight by  $\geq 50\%$ , EC values were not determined.

The no-observed-effect concentration (NOEC) was determined to be the highest concentration that caused no significant reduction of frond number or dry weight in comparison to the control. Williams' test ( $p \leq 0.05$ ) was used to determine significant effects after first checking the data for normality using Shapiro-Wilks' test and for homogeneity of variance using Bartlett's test.

12. **REPORTED RESULTS:** Initial measured concentrations averaged 98% of nominal (Table 3, attached). Terminal measured concentrations averaged 61% of nominal for the four highest concentration solutions. The lowest concentration solution contained dicamba at a concentration below the limit of detection. Therefore, results are based on initial measured concentrations. Recoveries of the 0- and 14-day quality control samples averaged 99% of nominal.

Frond counts for the control and the exposure concentrations after 14 days are given in Table 4 (attached). Plants exposed to the highest concentration of dicamba (3.8 mg ai/l) were slightly chlorotic and curled in comparison to the control. Plants in the intermediate three concentration solutions (1.9, 0.99, and 0.51 mg ai/l) were slightly chlorotic at test termination, and plants in the lowest concentration solution (0.25 mg ai/l) were normal. Based on frond number data, the 14-day  $EC_{50}$  and NOEC were determined to be  $>3.8$  and 0.25 mg ai/l, respectively. Based on the plant dry weight data (Table 5, attached), the 14-day  $EC_{50}$  and NOEC were determined to be  $>3.8$  and 3.8 mg ai/l,

respectively. Testing at a higher concentration range above the equivalency of the maximum application rate (2.9 mg ai/l) to develop an  $EC_{50}$  is not required by the test guidelines.

During the test, pH was 4.9-5.0 in all treatment and control solutions at test initiation and 6.1-6.2 at test termination. The temperature ranged from 23 to 27°C.

**13. STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:**

No conclusions were made by the study author.

The study director confirmed that this study was conducted in compliance with EPA Good Laboratory Practice (GLP) regulations (40 CFR Part 160) with the exception that maintenance of records on the test substance (stability, characterization, verification) is the responsibility of the sponsor. Additionally, routine water analyses were conducted at an independent laboratory that did not collect data in accordance with GLP procedures. A Quality Assurance statement was included in the report.

**14. REVIEWER'S DISCUSSION AND INTERPRETATION OF STUDY RESULTS:**

**A. Test Procedure:** The test procedure and the report were generally in accordance with the SEP and Subdivision J guidelines with the exception that the light intensity (3.2-4.8 klux) was lower than recommended (5 klux).

**B. Statistical Analysis:** Since the concentrations of test material in the terminal samples were all near 60% of nominal, the reviewer determined the mean measured concentrations of the test material. The mean measured concentrations were 0.20, 0.39, 0.77, 1.60, and 3.25 mg ai/l. The maximum amount of inhibition of frond number (occurring at the 3.25 mg ai/l level) and dry weight (occurring at the 0.39 mg ai/l level) was 18% and 2%, respectively. Therefore, the  $EC_{50}$  for duckweed growth based on both of these parameters is >3.25 mg ai/l.

The reviewer used analysis of variance coupled with Dunnett's test ( $p \leq 0.05$ ) to determine the lowest-observed-effect concentration (LOEC) and NOEC based on frond number (the most sensitive measure of dicamba effect). The results were similar to those of the author (see attached printout).

**C. Discussion/Results:** Although undissolved test material was observed in the primary stock solution, no mention of this was made regarding the test solutions.

Therefore, the reviewer believes that the material was dissolved when diluted with the nutrient solution, as evidenced by the near target nominal concentrations at test initiation.

This study is scientifically sound and meets the requirements for a Tier 2 aquatic plant growth and reproduction study. Based on mean measured concentrations of dicamba, the 14-day NOEC and LOEC for *L. gibba* were 0.20 and 0.39 mg ai/l, respectively. The 14-day EC<sub>50</sub> was >3.25 mg ai/l, which was greater than the equivalent of the maximum application rate.

D. Adequacy of the Study:

- (1) Classification: Core.
- (2) Rationale: N/A.
- (3) Repairability: N/A.

15. COMPLETION OF ONE-LINER: Yes, 7-27-93.

**Table 3. Concentrations of Dicamba measured in the exposure solutions during the 14-day static toxicity test with *Lemna gibba*.**

| Nominal Concentration (mg A.I./L) | Measured Concentration (mg A.I./L) <sup>a</sup> |           |                  |                 |
|-----------------------------------|---|-----------|------------------|-----------------|
|                                   | Day 0   | % Nominal | Day 14           | % Nominal       |
| 4.0                               | 3.8   | 95        | 2.7              | 69              |
| 2.0                               | 1.9   | 95        | 1.3              | 64              |
| 1.0                               | 0.99  | 99        | 0.55             | 55              |
| 0.50                              | 0.51  | 102       | 0.27             | 54              |
| 0.25                              | 0.25  | 101       | <0.14            | NA <sup>b</sup> |
| Control                           | <0.13   | NA        | <0.14            | NA              |
| QC #1 <sup>c</sup>                | 1.98<br>(2.00) <sup>d</sup>                     | 99.2      | 2.49<br>(2.50)   | 99.4            |
| QC #2                             | 0.970<br>(1.00)                                 | 97.0      | 0.991<br>(1.00)  | 99.1            |
| QC #3                             | 0.489<br>(0.500)                                | 97.7      | 0.256<br>(0.250) | 103             |

<sup>a</sup> Measured values are based on analytical results and not on rounded values (two significant figures) presented in this table.

<sup>b</sup> NA = Not Applicable

<sup>c</sup> QC = Quality Control sample

<sup>d</sup> Value in parentheses represents the nominal fortified concentration for the corresponding QC sample.

Springborn Laboratories, Inc.

**Table 4. Frond production and observations recorded for *Lemna gibba* after 3, 6, 9, 12, and 14 days exposure to Dicamba Technical.**

| Initial Measured Concentration (mg A.I./L) |                        | Fronds/replicate |         |                  |                        |                        |
|--|------------------------|------------------|---------|------------------|------------------------|------------------------|
|  |                        | Day 3            | Day 6   | Day 9            | Day 12                 | Day 14                 |
| 3.8  | A                      | 36               | 80      | 183              | 187                    | 368                    |
|  | B                      | 35               | 70      | 178              | 185                    | 349                    |
|  | C                      | 34               | 74      | 160              | 190                    | 313                    |
|  | Mean (SD) <sup>a</sup> | 35(1.0)          | 75(5.0) | 174(12)          | 187(2.5) <sup>bc</sup> | 343(28) <sup>bcd</sup> |
| 1.9  | A                      | 43               | 83      | 183              | 223                    | 352                    |
|  | B                      | 37               | 71      | 175              | 213                    | 355                    |
|  | C                      | 34               | 78      | 166              | 239                    | 373                    |
|  | Mean (SD) <sup>a</sup> | 38(4.6)          | 77(6.0) | 175(8.5)         | 225(13)                | 360(11) <sup>bd</sup>  |
| 0.99                                       | A                      | 41               | 86      | 194              | 237                    | 386                    |
|  | B                      | 36               | 88      | 179              | 222                    | 405                    |
|  | C                      | 44               | 84      | 191              | 252                    | 378                    |
|  | Mean (SD) <sup>a</sup> | 40(4.0)          | 86(2.0) | 188(7.9)         | 237(15)                | 390(14) <sup>bd</sup>  |
| 0.51                                       | A                      | 42               | 80      | 163              | 248                    | 365                    |
|  | B                      | 44               | 83      | 197              | 251                    | 378                    |
|  | C                      | 42               | 82      | 185 <sup>b</sup> | 233                    | 371                    |
|  | Mean (SD) <sup>a</sup> | 43(1.2)          | 82(1.5) | 182(17)          | 244(9.6)               | 371(6.5) <sup>bd</sup> |
| 0.25                                       | A                      | 36               | 75      | 177              | 227                    | 429                    |
|  | B                      | 39               | 80      | 210              | 260                    | 412                    |
|  | C                      | 41               | 88      | 191              | 246                    | 423                    |
|  | Mean (SD) <sup>a</sup> | 39(2.5)          | 81(6.6) | 193(17)          | 244(17)                | 421(8.6)               |
| Control                                    | A                      | 36               | 75      | 163              | 242                    | 406                    |
|  | B                      | 42               | 86      | 182              | 253                    | 416                    |
|  | C                      | 39               | 82      | 171              | 247                    | 432                    |
|  | Mean(SD) <sup>a</sup>  | 39(3.0)          | 81(5.6) | 172(9.5)         | 247(5.5)               | 418(13)                |

<sup>a</sup> Mean and standard deviation (SD) are calculated from original raw data not from rounded values presented in this table.

<sup>b</sup> Fronds were observed to be slightly chlorotic in comparison to control.

<sup>c</sup> Fronds were observed to be curled in comparison to control.

<sup>d</sup> Statistically different ( $p \leq 0.05$ ) as compared to the control based on Williams' Test.

Table 5. Dry frond weight (biomass) for *Lemna gibba* after 14 days exposure to Dicamba Technical.

| Initial<br>Measured<br>Concentration<br>(mg A.I./L) | Dry weight (g) |        |        |        | Mean (SD) <sup>a</sup> |
|---|----------------|--------|--------|--------|------------------------|
|   | Replicate      | A      | B      | C      |                        |
| 3.8   |                | 0.0756 | 0.0608 | 0.0588 | 0.0651(0.0092)         |
| 1.9   |                | 0.0759 | 0.0719 | 0.0984 | 0.0821(0.0143)         |
| 0.99  |                | 0.0749 | 0.0855 | 0.0804 | 0.0803(0.0053)         |
| 0.51  |                | 0.0533 | 0.0629 | 0.0755 | 0.0639(0.0111)         |
| 0.25  |                | 0.0864 | 0.0753 | 0.0891 | 0.0836(0.0073)         |
| Control   |                | 0.0583 | 0.0768 | 0.0611 | 0.0654(0.0100)         |

<sup>a</sup> SD=Standard deviation



lemna frond number  
 File: lem Transform: NO TRANSFORMATION

# ANOVA TABLE

| SOURCE         | DF | SS        | MS       | F      |
|----------------|----|-----------|----------|--------|
| Between        | 5  | 14916.278 | 2983.256 | 12.874 |
| Within (Error) | 12 | 2780.667  | 231.722  |        |
| Total          | 17 | 17696.944 |          |        |

Critical F value = 3.11 (0.05,5,12)  
 Since  $F > \text{Critical } F$  REJECT  $H_0$ : All groups equal

lemna frond number  
 File: lem Transform: NO TRANSFORMATION

## DUNNETTS TEST - TABLE 1 OF 2 Ho: Control < Treatment

| GROUP | IDENTIFICATION | TRANSFORMED<br>MEAN | MEAN CALCULATED IN<br>ORIGINAL UNITS | T STAT | SIG |
|-------|----------------|---------------------|--------------------------------------|--------|-----|
| 1     | control        | 418.000             | 418.000                              |        |     |
| 2     | 0.20           | 421.333             | 421.333                              | -0.268 |     |
| 3     | 0.39           | 371.333             | 371.333                              | 3.755  | *   |
| 4     | 0.77           | 389.667             | 389.667                              | 2.280  |     |
| 5     | 1.60           | 360.000             | 360.000                              | 4.666  | *   |
| 6     | 3.25           | 343.333             | 343.333                              | 6.007  | *   |

Dunnett table value = 2.50 (1 Tailed Value,  $P=0.05$ ,  $df=12,5$ )

*NOEL = 0.20 mg ai/l*  
*LOEL = 0.39 mg ai/l*

lemna frond number  
 File: lem Transform: NO TRANSFORMATION

## DUNNETTS TEST - TABLE 2 OF 2 Ho: Control < Treatment

| GROUP | IDENTIFICATION | NUM OF<br>REPS | Minimum Sig Diff<br>(IN ORIG. UNITS) | % of<br>CONTROL | DIFFERENCE<br>FROM CONTROL |
|-------|----------------|----------------|--------------------------------------|-----------------|----------------------------|
| 1     | control        | 3              |                                      |                 |                            |
| 2     | 0.20           | 3              | 31.073                               | 7.4             | -3.333                     |
| 3     | 0.39           | 3              | 31.073                               | 7.4             | 46.667                     |
| 4     | 0.77           | 3              | 31.073                               | 7.4             | 28.333                     |
| 5     | 1.60           | 3              | 31.073                               | 7.4             | 58.000                     |
| 6     | 3.25           | 3              | 31.073                               | 7.4             | 74.667                     |